

# Analysis of Hoechst Side Population (SP) Cells in Mouse Bone Marrow Using Low-Power UV Sources

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## Abstract

Discrimination of stem cells using flow cytometric analysis of Hoechst 33342 efflux by the ABCG2 transporter (termed the Hoechst "side population", or SP technique) is a valuable methodology for identifying bone marrow progenitor enriched for stem cells. Unfortunately, it requires a UV laser source on a large-scale cell sorter or benchtop analyzer. In previous work, we have demonstrated the utility of low-power near-ultraviolet laser diodes (NUVLDs) in analyzing the SP population on both cuvette and epifluorescence-based flow cytometers. In this study, we have experimentally determined the minimum UV power level required for the detection of the SP population on both types of cytometers using NUVLDs with a range of power levels, and using high-power UV-emitting LEDs in the epifluorescence-based system. A fiber-coupled NUVLD emitting at less than 3 mW gave adequate excitation for detection of SP on both cuvette and epifluorescence systems, and a focused UV LED gave resolution on the epifluorescence-based instrument approaching that of laser sources. These studies suggest that low levels of UV excitation, and non-coherent sources of UV light are useful for SP detection, permitting the design of low-cost analysis systems capable of analyzing this critical parameter.

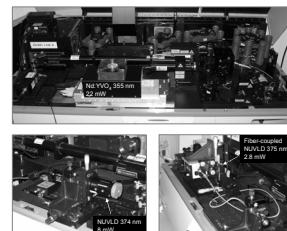
## Hoechst SP analysis using low-power UV lasers

Hoechst side population (SP) analysis is a critical technique for detecting stem cells and early progenitors. However, it requires a UV laser for Hoechst 33342 excitation, an uncommon and expensive laser wavelength not available on many flow cytometers. In this study, we have attempted to determine the minimum UV power level required to reliably visualize Hoechst SP, in the interest of designing inexpensive instrumentation for analyzing this important stem cell characteristic.

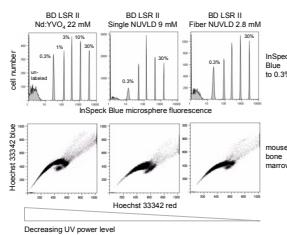
A series of UV-emitting lasers appropriate for benchtop analyzers (including Nd:YVO<sub>4</sub>, solid state and near-UV laser diodes) were assessed on a BD LSR II for their ability to visualize Hoechst SP in murine bone marrow. Near-UV laser diodes have been previously shown to be excellent sources of UV light for Hoechst SP analysis; in this study, a fiber-coupled NUVLD with a post-fiber emission of less than 3 mW was still able to visualize Hoechst SP with adequate resolution. The amount of UV excitation required for a cuvette-based flow cytometer therefore appeared to be minimal, and could be produced by a relatively inexpensive UV laser.

## Hoechst SP analysis on a low-cost flow cytometer

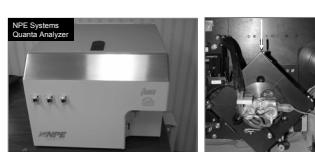
The Hoechst SP analysis shown above, while using low-cost UV laser sources, was still done on a BD LSR II, a costly polychromatic flow cytometer. Some of the above UV laser sources were therefore integrated into a NPE Quanta Analyzer, a low-cost flow cytometry platform with epifluorescence optics capable of accommodating a UV laser.



Above, Three laser systems of descending UV power, including a JDS Uniphase Lightwave Nd:YVO<sub>4</sub>, solid state (355 nm, 22 mW), a Coherent near-UV laser diode (374 nm, 8 mW) and a Point Source fiber-coupled NUVLD (374 nm, 2.8 mW post-fiber). All lasers were tested on a BD Biosciences LSR II.



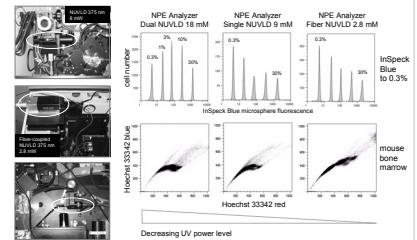
Above, Molecular Probes Invitrogen InSpeck Blue microspheres five fluorescent populations plus unlabeled, top row), and Hoechst SP in mouse bone marrow (bottom row), analyzed using the three laser sources above with descending power levels (22 to 2.8 mW) on a BD LSR II.



Above, The NPE Quanta Analyzer (NPE Systems, Inc.). The flow cell and stage are shown on the right. Excitation light (dotted arrow indicated by arrow) is reflected upwards through an epifluorescence objective, where it intercepts the cell stream through the flow cell. Fluorescence detection is by standard PMTs and bandpass filters/dichroics.

## Hoechst SP analysis on a low-cost flow cytometer using UV lasers

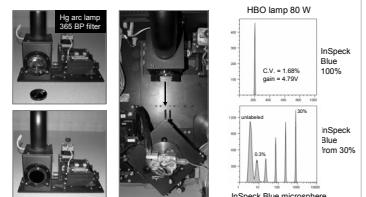
Near-UV laser diode sources with power levels ranging from 18 to less than 3 mW were integrated into the NPE Analyzer. Good Hoechst SP resolution was found using all sources, including the low-power fiber-coupled NUVLD.



Above left, NUVLD lasers mounted on the NPE Quanta. Above right, InSpeck Blue microsphere array and Hoechst SP in mouse bone marrow using NUVLD systems ranging from 18 to 2.8 mW in power level.

## Mercury arc lamp excitation on the NPE Analyzer

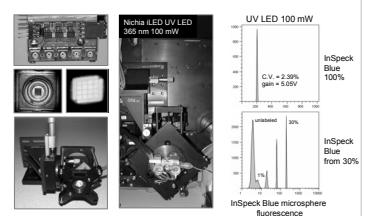
The NPE Analyzer is normally equipped with a 120 W Mercury arc lamp. This lamp, equipped with a UV bandpass filter, is a good UV source, allowing resolution of dim fluorescent signals approaching that of UV laser sources (based on a UV-excited bead array).



Above left and middle, the mercury arc lamp housing, alone and mounted on the NPE Quanta. Above right, InSpeck Blue 100% alone and 30%-to-unlabeled array using the mercury arc lamp.

## UV LED excitation on the NPE Analyzer

This instrument can also be equipped with a Nichia iLED UV LED, emitting at 365 nm with a power level of 100 mW. With the appropriate condenser optics, this UV source also gives relatively good microsphere resolution, although with poorer dim signal detection than the lamp.

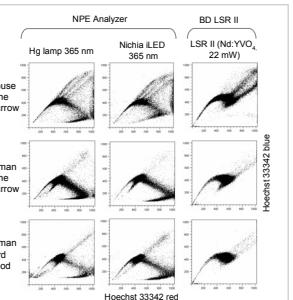


Above left and middle, an array of Nichia iLEDs, a close-up of one and its projected LED emission, in its X-Y carrier, and mounted on the MPE Quanta. Above right, InSpeck Blue 100% alone and 30%-to-unlabeled array using the iLED.

## Hoechst SP analysis using mercury arc lamp and UV LED excitation sources

Although their dim fluorescence resolution was lower than with UV laser sources, both mercury arc lamp and UV LED gave good Hoechst SP resolution.

Right, mouse bone marrow, human bone marrow and human cord blood analyzed on the NPE Quanta using mercury arc lamp (left column) or UV LED (middle column). PI viability fluorescence is visible on the Hoechst red axis. Hoechst SP analyzed on a BD LSR II using a Nd:YVO<sub>4</sub> solid state laser is shown for comparison (right column).



Hoechst SP analysis can be carried out using both very low power UV laser and non-laser sources, on simple inexpensive flow cytometric instrumentation. It should therefore be possible to design a flow cytometer for Hoechst SP at far lower cost than those currently available.